Séminaire de Microbiologie de Strasbourg

Jeudi 11 avril 2013
Forum de la Faculté de Médecine de Strasbourg
Salle 301

Programme

8h30 Accueil

8h55 Introduction, **Pr. Ermanno CANDOLFI**, *Institut de Parasitologie et de Pathologie Tropicale*

9h00 Conférence plénière

Modérateur : **Hugues de ROCQUIGNY**,
Laboratoire de Biophotonique et de Pharmacologie, Faculté de Pharmacie, Illkirch

Targeting host cell epigenetics : a novel mechanism of immune evasion by Toxoplasma gondii
Pr. Dr. Carsten LÜDER
Göttingen University Medical School, Dept of Medical Microbiology, GÖTTINGEN

10h00 Communications orales

Interactions between viral proteins from Human Papillomavirus and the ubiquitin-proteasome system
Juline POIRSON, *Equipe oncoprotéines, UMR 7242, IREBS, Illkirch*

The HIV-1 accessory protein Vpr counteracts CTIP2 in microglial cells
Sultan ALI, *Institut de Parasitologie et de Pathologie Tropicale, Department of Host-Pathogen Interaction (DHPI), Strasbourg*

Rôle des récepteurs Toll dans la transmission précoce de la Borréliose de Lyme
Quentin BERNARD, *EA 7290 : Virulence bactérienne précoce, Institut de Bactériologie, Strasbourg*

10h50 Session posters et pause-café offerte par la société Dutscher

11h15 Communications orales

***Pastrel* : a new *Drosophila* gene restricting infection by the picorna-like virus DCV**
Vincent BARBIER, *CNRS-UPR 9022, Institut de Biologie Moléculaire et Cellulaire, Strasbourg*

Role of extracellular apolipoprotein E in the HCV life cycle
Emilie CROUCHET, *Inserm U1110, Université de Strasbourg*

Syndecans mediate apolipoprotein E-hepatocyte cell surface interactions during hepatitis C virus entry
Mathieu LEFEVRE, *Inserm U1110, Université de Strasbourg*

Redox-active 1,4-naphthoquinones in a cascade of NADPH-consuming bioactivation – A new strategy to combat malarial parasites
Katharina EHRHARDT, *European School of Chemistry, Polymers and Materials (ECPM), University of Strasbourg, UMR CNRS 7509 and Department of Infectiology, University of Heidelberg, Germany*

12h45 Session posters et cocktail déjeunatoire

14h00 Conférence plénière

Modérateur : **Dr Gilles PREVOST,**
EA-7290, Virulence bactérienne précoce, FMTS UDS, Institut de Bactériologie, Strasbourg

A metagenomic insight into ou gut's microbiome
Dr. Patricia LEPAGE,
Micalis – Building 405, Domaine de Vilvert, INRA, 78350 JOUY-EN-JOSAS

15h00 Communications orales

Essential residues for Panton – Valentine leucocidin binding : inhibition by using Ala-scannin mutagenesis
Benoit-Joseph LAVENTIE, *EA-7290 Virulence bactérienne précoce, FMTS, Université de Strasbourg – CHRU Strasbourg.*

Cell biology investigation of the pyochelin pathway in *Pseudomonas aeruginosa*
Olivier CUNRATH, *Université de Strasbourg, UMR 7242 - Transport membranaire bactérien, Pôle API, Illkirch.*

Ribosomal protein S1 unfolds structured mRNAs on the ribosome for translation initiation in *Escherichia coli*
Mélodie DUVAL, *Architecture et Réactivité de l'ARN, UPR 9002, Université de Strasbourg, CNRS, Institut de Biologie Moléculaire et Cellulaire, Strasbourg*

15h50 Session posters et pause-café offerte par la société Thermo-Fischer

16h15 Communications orales

La vie face à l'arsenic : étude du génome et de la physiologie de la bactérie autotrophe arsénite-oxydante *Rhizobium* sp. NT-26
Jérémy ANDRES, *Laboratoire de Génétique Moléculaire, Génomique et Microbiologie, UMR 7156 CNRS-Université de Strasbourg*

Effets photocatalytiques du TiO_2 sur les lipides et les protéïnes *d'E. coli*.
Gaëlle CARRE, *Laboratoire de Biophonique et Pharmacologie, UMR 7213, Institut de Chimie et Procédés pour l'Energie, l'Environnement et la Santé, UMR 7515 CNRS/UdS.*

Genetic basis of reproductive isolation in *Saccharomyces cerevisiae*.
Jing HOU, *Laboratoire génétique moléculaire, génomique et microbiologie, UMR 7156, Strasbourg*

REMISE DU PRIX de la meilleure communication orale, Dr. Valérie GEOFFROY

17h05 CONCLUSIONS, Dr. Philippe ANDRE, *Laboratoire de Biophotonique et Pharmacologie, Faculté de Pharmacie*

Targeting host cell epigenetics: A novel mechanism of immune evasion by *Toxoplasma gondii*

Carsten Lüder

Institute for Medical Microbiology, University Medical School, Georg-August-University Göttingen, Germany

Toxoplasma gondii is a common unicellular parasite of humans and other vertebrates and can lead to overt disease mostly in immune-suppressed patients or in fetuses. Since the parasite life cycle critically depends on establishing a persistent infection, evasion of immunity by *T. gondii* represents a major factor of pathogenic fitness. By using genome-wide expression profiling we showed recently that parasite infection renders murine macrophages globally unresponsive to stimulation with IFN-g, i.e. the major mediator of resistance against *T. gondii*. Analysis of the underlying mechanisms revealed that *Toxoplasma* interferes with the assembly of chromatin remodelling complexes at IFN-g-responsive DNA sequences. Furthermore, the acetylation of histones at IFN-g-regulated promoters was found to be severely impaired. Likewise, histone modifications at LPS-triggered promoters were also inhibited by *T. gondii*. These results indicate that epigenetic silencing and activation of immune response genes may represent a previously unrecognized mechanism of *T. gondii* which impairs host immunity against the parasite. Importantly, treatment with histone deacetylase inhibitors can rescue *Toxoplasma*-infected macrophages from the inability to respond to IFN-g. Implications of these novel findings will be discussed.

THE HUMAN INTESTINAL MICROBIOME ; Interactions with host and dysfunctions

Patricia Lepage
INRA, UMR1319-MICALIS, Functionality of the Intestinal Ecosystem, Jouy-en-Josas, France

A complete understanding of the human biology will only be fully assessed by combining the analysis of the host and its surrounding environment. The human gastrointestinal tract hosts more than 100 trillion bacteria and archaea, which together make up the gut microbiota. Even though the amount of bacteria in the human gut outnumber human cells by a factor of 10, some finely tuned mechanisms allow these microorganisms to colonize and survive within the host in a commensalism relationship. The human gut microbiota can be considered an organ within an organ that co-evolved with humans to achieve a symbiotic relationship leading to physiological homeostasis. The human host provides a nutrient-rich environment and the microbiota provides indispensable functions that humans cannot exert themselves. It plays a beneficial role in the metabolism of non-digestible food components (dietary fibers), in producing vitamins and short-chain fatty acids and shaping host physiology.

Chaotic in the early stages of human life, the assembly of the human gut microbiota remains globally stable over time in healthy conditions in the absence of perturbation. The average total number of bacterial species was estimated to be close to 1000 per individual. The restricted number of phyla in comparison to other ecosystems has suggested a tight co-evolutionary history between the host and its microbiota. Remarkably, shifts in the bacterial makeup of the human gut microbiota, also called dysbiosis, have been associated with digestive tract dysfunctions such as inflammatory bowel disease (IBD), irritable bowel syndrome (IBS) and obesity.

Yet, further insights into the human gut ecosystem are needed to comprehend the exact role of microbiota in health and disease. Because most of the bacteria inhabiting the gut are uncultivable, their functions cannot be inferred from composition data. Today, *via* the metagenomics approach, the bacterial genomic content of an ecosystem can directly be accessed from the environment, without any cultivation step. The MetaHit European consortium has hence proposed the existence of a common bacterial core composed of 75 bacterial species. Nonetheless knowing which microbes are there is not sufficient. Meta-omics (from metagenomes to metabolomes) has been developed to answer essential questions such as ‘What is the genetic potential of the non-cultured bacterial fraction of the gut microbiota?’, ‘What are these microbes really doing?’ and finally “How they interact with their host?.

Interactions between viral proteins from Human Papillomavirus and the ubiquitin-proteasome system

Juline Poirson[1], **Marie-Laure Straub**[1], **Patricia Cassonnet**[2], **Yves Jacob**[2], **Murielle Masson**[1]

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[2] *Unité Génétique, Papillomavirus et cancer humain, Institut Pasteur, Paris*

The human papillomavirus (HPVs) are the archetype of DNA oncogenic viruses. Nowadays more than 180 HPVs different genotypes are identified, only a few are closely associated with the development of cancer. It is mainly the high-risk mucosal HPVs belonging to the alpha genus including the HPV16 and HPV18, which are involved in many cancers. These high-risk mucosal HPVs are implicated in 95% of cervical cancer, the second most prevalent cancer in women worldwide. Despite the recent elaboration of vaccination, HPV infection is still an important public health problem. Some HPVs from the beta genus, involved in skin lesions, appear to be implicated in the development of non-melanoma skin cancer and in epidermodysplasia verruciformis defining a group of high-risk cutaneous HPVs.

The ubiquitin-proteasome system (UPS) is a major route of degradation of intracellular proteins in eukaryotes. HPV E6 and E7 proteins hijack the UPS to target a number of important negative regulatory proteins for degradation, which contribute to carcinogenesis.

Our study aim is to dissect the multiple interactions between E6 and E7 from mucosal and cutaneous HPV types and UPS effectors and identify novel functional mechanisms. The first part is focused on a mechanistic study of the hijacking of E6AP (an E3 ubiquitin ligase) by HPV16E6. The second aspect consists of identifying novel UPS effectors interacting with E6 and E7 from mucosal and cutaneous HPVs. It relies on a systematic screening of a library of UPS effectors with a novel protein-protein interaction assay (PCA) (Cassonnet *et al.* (2011) Nature Methods). This approach is based on the complementation of *Gaussia princeps* luciferase. Finally, the UPS effectors necessary for the proliferation of alpha or beta immortalised human keratinocytes will be identified by screening a siRNA library dedicated to the UPS.

Cassonet, P., Rolloy, C., Neveu, G., Vidalain, P.-O., Chantier, T., Pellet, J., Jones, L., Muller, M., Demeret, C., Gaud, G., Vuillier, F., Lotteau, V., Tangy, F., Favre, M. & Jacob, Y. (2011) Benchmarking a luciférase complémentation assay for detecting protein complex. *Nature Methodes* **8** (12), 990-992.

résumé SMS 2013: présentation orale

"The HIV-1 accessory protein Vpr counteracts CTIP2 in microglial cells"

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Usurping the host ubiquitination proteasome system (UPS) to inactivate the undesirable host protein is a common viral strategy. HIV-1 proteins inactivate the detrimental host proteins by this system. In Microglial cells, CTIP2 represses both initial phase and late phase of HIV-1 gene transcription. As HIV-1 can still replicate in the presence of CTIP2, we postulated that it might inactivate CTIP2 by using Cul4 E3 ubiquitin ligase complex to resume its replication.

We observed higher CTIP2 expressions in the absence of Vpr, with no effect on CTIP2 mRNA and proteasome inhibitor can block this degradation. Co-immunoprecipitation assays showed that CTIP2 is associated with DCAF1 and DDB1 in the absence and presence of Vpr. We showed that this degradation is prevented by the using Vpr mutant (Q65R) and by knock down of DCAF1. Finally, we observed the co-localization of CTIP2 with Cul4A-DCAF1-DDB1 complex even in the absence of Vpr, in microglial cells. Additionally, DCAF1 interacts with CTIP2-associated heterochromatin enzymes complex.

Our results suggest that Vpr expression increases the turnover of CTIP2 in HIV-1 productively infected cells. By degrading CTIP2, HIV-1 counteracts CTIP2-mediated silencing of its expression and favors its replication.

Rôle des récepteurs Toll dans la transmission précoce de la Borréliose de Lyme.

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La maladie de Lyme est une infection bactérienne, due à des spirochètes du groupe *Borrelia burgdorferi* sensu lato et transmises par des tiques du genre *Ixodes*. Elle constitue la maladie à transmission vectorielle la plus répandue dans l'hémisphère Nord. Lors de la phase primaire de la maladie, il se développe au point de piqûre dans la plupart des cas une inflammation cutanée : l'érythème migrant.

L'interface cutanée joue un rôle important dans la transmission de *Borrelia,* puisque la bactérie y est inoculée ainsi que la salive de tique. De plus, une réponse immunitaire contre la bactérie y est initiée. Les cellules résidentes de la peau, notamment les kératinocytes de l'épiderme, prennent part à cette initiation en reconnaissant la lipoprotéine de surface OspC de *Borrelia* grâce au récepteur Toll hétérodimérique 2/1 (TLR2/1)[1]. Cette reconnaissance s'effectue dans un contexte de blessure cutanée puisque les pièces piqueuses de la tique produisent une dilacération de la peau. La blessure pourrait activer l'inflammation via le récepteur TLR3 capable de détecter la libération d'ARN par les cellules nécrotiques issues de la blessure[2]. L'hypothèse principale de ce travail est que *Borrelia* pourrait utiliser à son avantage d'autres récepteurs Toll activés durant la transmission de *Borrelia*.

Par une approche *in vitro* sur kératinocytes humains primaires, l'inflammation liée à la lésion est modélisée par l'activation de TLR3 par le ligand Poly:IC, puis nous ajoutons la protéine OspC de *Borrelia*, ligand de TLR2 afin de mesurer l'interaction TLR3-TLR2. Nous observons ainsi que la protéine OspC lipidée est capable d'inhiber significativement l'inflammation induite par le Poly:IC. Cette inhibition se caractérise par une baisse de l'expression de cytokine pro-inflammatoire (TNF-α), de chimiokines (IL-8, MCP-1) et de peptides antimicrobiens (hBD2, Rnase7). Lorsque la lipoprotéine totale est remplacée dans l'activation de TLR2 par la partie lipidique seule (Pam3CSK4), l'inhibition de l'inflammation persiste. En revanche, la partie protéine seule a peu ou pas d'effet, soulignant le rôle de la partie lipidique dans l'inflammation. *Borrelia* pourrait ainsi utiliser de multiples TLRs afin de faciliter sa transmission à l'hôte vertébré.

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2) Kariko´, K., H. Ni, J. Capodici, M. Lamphier, and D. Weissman. (2004) mRNA is an endogenous ligand for Toll-like receptor 3. *J. Biol. Chem*. **279**, 12542–12550.

Pastrel: a new *Drosophila* gene restricting infection by the picorna-like virus DCV.

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Since the discovery of the evolutionarily conserved TOLL and IMD pathways, involved in anti-fungal and anti-bacterial immune responses, the fruit fly *Drosophila melanogaster* is used as a model to study the molecular mechanisms of innate immunity. To defend against viral pathogens, *Drosophila* relies on two main facets: the RNA interference (RNAi) pathway, which plays a broad role in the control of viruses, and virus specific inducible responses (Kemp et al, 2013). We also observed that the fly genetic background can modulate the resistance to infection by Drosophila C Virus (DCV), a natural pathogen of *Drosophila*. A genome wide association study recently showed that polymorphisms in the gene named *pastrel*, localized on the left arm of the third chromosome, affect the resistance to DCV infection (Magwire et al, 2012). We are now deciphering the role of this uncharacterized gene in the control of DCV infection. Our loss of function and gain of function experiments indicate that *pastrel* encodes a molecule opposing DCV infection, raising the question of the mechanism involved. Co-localization experiments indicate that Pastrel is localized in lipid droplets. All together, our data suggest a connection between lipid droplets and restriction of viral infection in *Drosophila*, as already described in mammals between the restriction factor Viperin, present on lipid droplets, and the replication of the human pathogen Hepatitis C Virus (Helbig et al, 2011).

Bibliography

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3. Magwire, M. M. *et al.* Genome-Wide Association Studies Reveal a Simple Genetic Basis of Resistance to Naturally Coevolving Viruses in Drosophila melanogaster. *PLoS Genet* **8,** e1003057 (2012).

Role of extracellular apolipoprotein E in the HCV life cycle

Emilie Crouchet[1,2], Mathieu Lefèvre[1,2], Thomas F. Baumert[1,2,3] and Catherine Schuster[1,2]

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Hepatitis C virus (HCV) infection is a major cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma worldwide. Many studies have shown that HCV life cycle and lipid metabolism are closely linked. Indeed, the virus circulates in the blood of infected patients associated with lipoproteins to form an infectious lipoviroparticle (LVP). Apolipoprotein E (apoE) is present at the LVP surface and plays a crucial role in HCV entry, assembly and egress. In the blood, this protein is also found in a lipoprotein associated form or a lipid-free form. Both forms are actively recycled by hepatocytes, following receptor-mediated endocytosis. Using HCV cell culture model system (HCVcc), we aimed to study the role of extracellular apoE and its recycling process in the HCV life cycle.

We demonstrated that extracellular addition of free apoE or apoE loaded very low-density lipoprotein (VLDL) decreases markedly viral replication and infectivity in a dose-dependent manner. Moreover, a silencing of Rab11, a Rab GTPase involved in apoE recycling, doesn't restore viral replication suggesting that apoE acts on the HCV life cycle independently of its recycling process. To understand interactions between apoE and the cell surface, we used the heparinase 1 which cleaves the heparan sulfate proteoglycans (HSPGs). We showed that HSPGs are essential for apoE-mediated decrease of HCV replication. Finally, using western blotting, we observed that addition of apoE in cell culture media modulates dose-dependently the expression of the low-density lipoprotein receptor (LDLR), an apoE receptor, the tetraspanin CD81, an HCV receptor, and the apolipoprotein A1 (apoA1), a protein associated with the high density lipoproteins (HDL).

All these results suggest that apoE activates a signaling pathway, which regulates hepatocytes lipid metabolism and thus decreases HCV replication and production. Identification of this signaling pathway is under investigation. Understand the involved mechanism could help us to find new therapeutic targets.

Syndecans mediate apolipoprotein E-hepatocyte cell surface interactions during hepatitis C virus entry

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Background: In hepatitis C virus (HCV) infected patients, virions are associated with VLDL-type lipoproteins forming an infectious lipo-viro-particle (LVP). Apolipoprotein E (apoE), which is an integral component of Very Low Density Lipoprotein (VLDL), has been shown to play a crucial role in HCV life cycle. Using a trans-complementation assay and a RNAi screen we aimed to identify the cellular targets of virion-associated apoE on the hepatocyte cell surface. **Methods:** The functional role of apoE and its cellular targets was investigated in a trans-complementation assay. Endogenous apoE was first silenced in Huh7.5.1 cells with a specific siRNA. ApoE expression was then restored using recombinant adenoviral vectors. The functional effect of apoE wild-type (wt) and mutant constructs was then investigated using HCV cell culture (HCVcc) model system. We focused on the functional analysis of the heparan sulfate proteoglycan (HSPG) high affinity domain (HSPG-BD), which overlaps with the well defined apoE receptor binding domain. **Results:** We demonstrated that apoEwt adenoviral transduction restored HCVcc infectious particle production in apoE depleted cells. In contrast, apoE deleted from the HSPG-BD leads to capsid-free HCVcc production suggesting a role of this domain in HCV assembly. Moreover, double alanine substitutions in the apoE HSPG-BD, apoE K143A, K146A or apoE R142A, R145A lead to regular HCVcc production but failed to restore HCVcc infectivity suggesting a role of apoE HSPG-BD in HCV entry. To confirm the role of the apoE-HSPG-BD in HCV entry, we designed an apoE-derived peptide overlapping the HSPG-BD. This peptide inhibits HCVcc binding in a dose-dependent manner. Since syndecans are members of the HSPG family, we next investigated their role in HCV binding and entry. Using a RNAi screen targeting the individual members of the syndecan family, we identified the syndecans specifically involved in HCV entry. **Conclusion:** HCV binding and entry are mediated by apoE-HSPG interactions and required cell surface expression of defined members of the syndecan family. These results advance our understanding of the very first steps of virus-host interactions and are relevant for the development of antivirals and vaccines targeting HCV entry.

Redox-active 1,4-naphthoquinones in a cascade of NADPH-consuming bioactivation – A new strategy to combat malarial parasites

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Malaria still remains one of the most important infectious diseases worldwide. The emergence and spread of drug resistance against most used antimalarials raises an urgent need for new therapeutics. Our previous work in medicinal chemistry led to the selection of 1,4-naphthoquinone (benzylNQ) derivatives with potent antiparasitic activity against the blood stages of the human pathogen *Plasmodium falciparum* in culture and against *Plasmodium berghei* in infected mice.[1] Antimalarial benzylNQ are thought to be bioactivated in infected red blood cells through a cascade of redox reactions (prodrug effect), involving both NADPH-dependent glutathione reductases of infected host cells, resulting in conversion of methemoglobin(Fe^{III}), the major nutrient of the parasite, to indigestible hemoglobin(Fe^{II}). Ultimately, antimalarial benzylNQ are suggested to decrease the hemozoin formation and to affect the cellular redox equilibrium, resulting in parasite development arrest.

In vitro studies with *Plasmodium* parasites support the high potential of benzylNQ as potent antimalarial agents. With respect to drug safety, the compounds do not trigger hemolysis or cytotoxicity against human cells. Recent data showed that benzylNQ derivatives are active at nanomolar concentrations against ring stages and various field strains of *P. falciparum* from different countries irrespective of their resistance towards used antimalarials such as chloroquine and quinine. With respect to induction of drug resistance, evidence for a very low potential was assessed on cultured blood stages under drug pressure, both at 4-fold I_{50} and 4-fold I_{90} concentrations of the most active benzylNQ. Furthermore, by using transgenic *P. falciparum* parasites resistant to inhibitors of the mitochondrial electron transport chain, we were able to show that the lead benzylNQ does not share a common mode of action with the antimalarial atovaquone.[2] These data confirm the high potential of the series of redox-active benzylNQ as antimalarial compounds with a novel mode of action.

[1] Müller T, *et al.* Glutathione reductase-catalyzed cascade of redox reactions to bioactivate potent antimalarial 1,4-naphthoquinones – a new strategy to combat malarial parasites. *J Am Chem Soc* 2011, 133:11557-71.

[2] Ehrhardt K, *et al.* The antimalarial activities of methylene blue and the 1,4-naphthoquinone 3-[4-(trifluoromethyl)benzyl]-menadione are not due to inhibition of the mitochondrial electron transport chain. Antimicrob Agents Chemother. 2013 Feb 25, in press.

Essential residues for Panton - Valentine leucocidin binding: inhibition by using Ala-scanning mutagenesis

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The bipartite Panton-Valentine Leucocidin (PVL composed of LukS-PV and LukF-PV) is the major staphylococcal leucocidin involved in severity of some necrotizing infections. Albeit both components get a specific binding onto target cells, mostly PMNs, monocytes and macrophages, LukS-PV determines the early event leading to the cell activation, and to the pore formation. In this study, we aimed to uncover the LukS-PV binding domain, because of its priority and its greater affinity to only complex membranes; Nineteen mutations were driven by Alanine-scanning priority and its greater affinity to only complex membranes; Nineteen mutations were driven based on amino acid function, exposure, conservation between LukS-PV related compounds, and located on two loops of the « Rim » domain essential for LukS-PV binding. Mutations affecting R73, Y184, R242, H245, Y246, Y250 led to proteins having 13 to >200 times lowered affinities. Thrre-dimensional structures of the most deficient mutants showed that their rim folding remains conserved compared to wild-type LukS-PV. These amino acids form a molecular surface exposed to solvents at the bottom of LukS-PV. Except Y184 which is specific in LukS-PV, this spatial cluster is conserved ionly into HlgC, amongst bipartite leucotoxins, and suggests an essential structural organization.

Reference: Benoit-Joseph Laventie, Frédéric Guérin, Lionel Mourey, Laurent Maveyraud, Gilles Prévost. Essential residues for Panton Valentine Leukocidin S component binding to cellular membranes. Journal of Biological Chemistry, 2013, *in press*.

Cell biology investigation of the pyochelin pathway

in *Pseudomonas aeruginosa*

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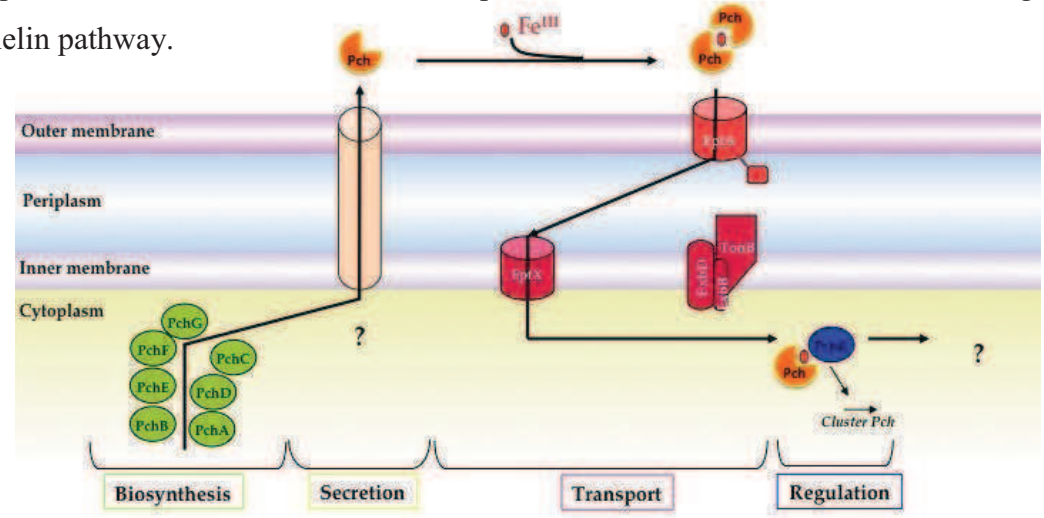
Pseudomonas aeruginosa is an opportunisic pathogen, able to infect human. It produces two major siderophores, pyoverdine and pyochelin, to cover its need in iron (FeIII). The structure of pyocheline was established as (4'*R*, 2"*R*, 4"*R*)-2'-(2-hydroxyphenyl)-3"-methyl-4',5',2",3",4",5"-hexahydro-[4',2"]bithiazolyl-4"-carboxylic acid with three chiral centers. The last 20 years, studies on this siderophore have been mostly focused on the biosynthesis pathway and the import of ferri-pyochelin throw the outer and inner membranes, but the subcellular distribution of all these proteins in particular areas of the cytoplasm or within the membrane has never been investigated. In additions, the proteins involved in secretion of pyochelin across the inner and outer membranes still stay unidentified. Here we used chromosomal replacement to generate *P. aeruginosa* strains producing fluorescent fusions with PchA and PchE, proteins involved in the biosynthesis. Epifuorescence microscopy imaging showed that PchA-mCHERRY and PchE-mCHERRY had mainly a polar localisation. Cellular fractionation showed that PchA-mCHERRY was partialy associated to the inner membrane in contarst to PchE-mCHERRY which is totaly cytoplasmic. The description of the localisation of theses proteins contributes to the understanding of the pyochelin pathway.



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Ribosomal protein S1 unfolds structured mRNAs on the ribosome for translation initiation in *Escherichia coli*

Mélodie Duval, Alexey Korepanov, Olivier Fuchsbauer, Pierre Fechter, Andrea Haller, Attilio Fabbretti, Ronald Micura, Bruno Klaholz, Mathias Springer, Pascale Romby and Stefano Marzi

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Regulation of gene expression is crucial for life as it increases the adaptability of any organism. Bacteria can modify their growth to various environments in their living conditions by changing and coordinating the synthesis of defined proteins. Regulation is exerted at many steps from transcription, translation, messenger RNA (mRNA) stability, to post-translational modification of proteins. Translation is mainly regulated at the initiation step, where different mechanisms of activation or repression can take place. Specific sequences or structure elements in the 5' untranslated regions of mRNAs are often involved to modulate the accessibility of the ribosome. It has been described that structured mRNAs interact with the ribosome in a two-step pathway: the mRNA first anchors on the platform of the 30S small ribosomal subunit and then moves into the mRNA channel to promote the codon-anticodon interaction. We show that a particular ribosomal protein, r-protein S1, confers to the small ribosomal subunit an RNA chaperone activity, which unfolds specific mRNA structures allowing their translation. Besides, we show that r-protein S1 is not required for all mRNAs and acts differently in translation according to the signals present in the mRNA. Hence, r-protein S1 confers specific activities to the ribosome that are adjusted to a given mRNA to create tunable initiation complexes to selectively translate unstructured or structured mRNAs.

Key words: ribosome, translation initiation, structured mRNA, RNA chaperone

La vie face à l'arsenic : étude du genome et de la physiologie de la bactérie autotrophe arsénite-oxydante *Rhizobium* sp. NT-26

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L'arsenic est largement répandu dans l'environnement et sa présence peut-être le résultat de processus naturels ou d'activités anthropiques. Les micro-organismes ont développé différentes stratégies pour faire face à des composés toxiques tels que l'arsenic, notamment en mettant en place des processus de résistance ou en les métabolisant. Par des expériences de physiologie et des approches dites « omiques », cette étude vise à mieux comprendre les déterminants génétiques de la réponse au stress arsénié chez une Alphaproteobactérie isolée d'une mine d'or contenant de l'arsenic: *Rhizobium* sp. NT-26. Bien que phylogénétiquement apparentée aux bactéries associées aux plantes, cet organisme a perdu les capacités colonisatrices nécessaires à la symbiose tout en gagnant les fonctions permettant son maintien dans sa niche écologique. En effet, le génome de *Rhizobium* sp. NT-26 comprend un mégaplasmide contenant les différents gènes qui lui permettent de métaboliser l'arsénite et de résister à d'autres métaux. De manière remarquable, une régulation coordonnée des mécanismes d'oxydation de l'arsénite et de la mobilité/formation de biofilm a été observée bien que les gènes nécessaires à ces processus sont respectivement portés par le mégaplasmide et le chromosome. Pris ensemble, ces processus illustrent l'impact que peut avoir l'environnement sur l'évolution des génomes bactériens, et notamment l'amélioration de l'aptitude des souches bactériennes à s'adapter grâce à l'acquisition de nouvelles fonctions.

Effets photocatalytiques du TiO_2 sur les lipides et les protéines d'*E.coli*

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La photocatalyse hétérogène est un procédé d'oxydation avancée utilisé dans de nombreuses applications telles que la décontamination de l'eau, de l'air et des surfaces. Cette technologie permet la dégradation de la plupart des polluants chimiques et biologiques. Le catalyseur souvent utilisé est le dioxyde de titane (TiO_2) qui exposé aux UV-A peut générer des Reactive Oxygen Species (ROS). Ces ROS peuvent induire une inactivation des microorganismes notamment *via* l'oxydation des constituants essentiels de la cellule. Dans le cadre de cette étude, nous nous sommes intéressés à l'impact de l'effet photocatalytique sur les lipides membranaires et le protéome d'*E.coli*. Les tests photocatalytiques ont été réalisés en phase liquide pour différentes concentrations en TiO_2 P25 (Evonik), photocatalyseur de référence, et pour différents temps d'exposition sous irradiance UV-A de 30 W/m^2.

L'influence de la photocatalyse sur la peroxydation lipidique a été évaluée à l'aide du kit OxiSelect TBARS Assay. Nous avons montré qu'après 60 min sous irradiation UV-A, l'action photocatalytique du TiO_2 à 0,4 g/L était associée à un triplement significatif de la peroxydation lipidique. De même, le fait de piéger $O_2^{\circ-}$ *via* l'ajout de Superoxyde Dismutase induit une réduction significative de cette peroxydation. Nous avons pu conclure qu'$O_2^{\circ-}$ est un des principaux ROS induisant la peroxydation lipidique lors de l'action photocatalytique.

En parallèle, le protéome d'*E.coli* a été étudié par électrophorèse 2D en gels SDS-PAGE, après traitement photocatalytique de 30 min en présence de TiO_2 à 0,1 et 0,4 g/L. Les protéines correspondant aux principaux spots modifiés ont été analysées par spectrométrie de masse (LC-MS), et au total une vingtaine de spots ont été sélectionnés. L'effet cytotoxique du TiO_2 non éclairé est non négligeable puisqu'en présence de 0,1 et 0,4 g/L, 7 et 19 spots ont respectivement disparu par rapport au gel témoin. Sous l'action photocatalytique, 7 et 3 protéines supplémentaires ont été modifiées pour des concentrations en TiO_2 respectives de 0,1 et 0,4 g/L. Après identification par LC-MS, nous avons établi que la majorité des protéines dégradées sont impliquées dans la réponse au stress oxydatif.

En conclusion, nous avons montré que la production de ROS $O_2^{\circ-}$ est la principale responsable de l'action photocatalytique du TiO_2 sur *E.coli* et conduit aux phénomènes de peroxydation lipidique et à la dégradation de protéines régulant le stress oxydatif.

Genetic basis of reproductive isolation in *Saccharomyces cerevisiae*

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Identification of the genetic variation between individuals within a species is valuable to understanding the patterns of molecular evolution. In all species, genetic diversity is the raw material for phenotypic diversity upon which the natural selection acts. At the inter-specific level, such genetic diversity including sequence divergence, Dobzhansky-Muller genetic incompatibilities and chromosomal rearrangements could result in reproductive isolations (e.g. reduced offspring) thus promotes the formation of new species. Nevertheless, expanding evidence shows when crossing individuals from a same species, offspring may sometimes have reduced viability. To date, the genetic basis of such intra-specific reproductive isolation within the yeast *Saccharomyces cerevisiae* remains largely elusive.

To identify cases of reproductive isolation within *S. cerevisiae*, we looked at a large collection of strains sampled from different ecological niches (beer, bread, vineyards, immunocompromised individuals, various fermentations and nature) and from locations on different continents. We performed pairwise crosses and then measured meiotic sterility in diploids. Most strains are compatible showing a high frequency of viable spores (around 90%). However, we found that crosses between some *S. cerevisae* strains produce a low frequency of viable spore: 75%, 50% and 1%, indicating the presence of post-zygotic isolation. Using different mapping strategies, we discovered a non-equilibrium reciprocal translocation that repeatedly occurs in several strains, which is responsible for the observed decrease of spore viability of 75%. Fine scale analysis of the translocation sites and the gene expression data suggests that environmental adaptation and natural selection played a pivotal role for the observed translocation.

This study shows how natural selection acts upon phenotypic traits and shapes the genome. It gives a first insight into the origin and molecular mechanisms responsible for the onset of reproductive isolation within a yeast species.

* Presenter.

The efflux pump PvdRT-OpmQ and its role in iron uptake by the siderophore pyoverdine in Pseudomonas aeruginosa

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Pseudomonas aeruginosa secretes the fluorescent siderophore, pyoverdine, to enable iron acquisition. This molecule is composed of a fluorescent chromophore linked to an octapeptide and chelates iron with a high affinity. Three genes (*pvdR, pvdT and opmQ*) with the potential to encode an efflux system are adjacent to, and coregulated with genes required for pyoverdine-mediated iron transport.

Epifluorescence microscopy and cellular fractionation were used to investigate the role of the efflux pump, PvdRT-OpmQ, in pyoverdine secretion. Bacteria lacking this efflux pump accumulated pyoverdine, or a fluorescent precursor, in the periplasm, due to their inability to efficiently secrete into the media newly synthesized pyoverdine (1). These data suggested that PvdRT-OpmQ is involved in secretion of newly synthesised pyoverdine. With the same approached we showed that PvdRT-OpmQ is also involved in the secretion of pyoverdine having already transported iron into bacteria (recycling of pyoverdine) (2).

At last, uptake assays with various outer membrane transporter FpvA and PvdRT-OpmQ mutants, monitored by inductively coupled plasma-atomic emission spectrometry (ICP-AES) for metal detection, and by fluorescence for pyoverdine detection, showed that PvdRT-OpmQ also plays a key role in the control of the metal selectivity of the pyoverdine pathway (3,4). It excretes from the bacterial periplasm any unwanted pyoverdine-metal complex transported by the outer membrane transporter FpvA.

The pyoverdine pathway will be discussed in the light of all these biological functions of PvdRT-OpmQ.

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Une nouvelle approche génétique pour la sélection rapide et efficace de cellules haploïdes chez *Saccharomyces kluyveri*

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L'identification des variations génétiques entre individus d'une même espèce est essentielle afin d'avoir une compréhension approfondie des mécanismes à l'origine de l'évolution et des différences de phénotypes observées. La diversité génétique est la matière première de la variation phénotypique et présente un intérêt pour l'adaptation de tout être vivant à son environnement.

Dans ce contexte, l'équipe s'intéresse à l'étude des relations entre génotype et phénotype notamment au sein d'une espèce de levure appartenant au phylum des hémiascomycètes : *Saccharomyces kluyveri.* Cet eucaryote unicellulaire représente un excellent modèle d'étude et se prête aisément à de nombreuses études génétiques basées sur la possibilité de croiser deux souches haploïdes stables, de signes sexuels opposés (*MAT*a et *MAT*alpha) sur la base de leur diversité génétique et phénotypique pour former un zygote. La cellule diploïde peut alors être induite à se diviser par méiose en quatre spores haploïdes. La détermination du signe sexuel des cellules haploïdes obtenues se fait habituellement par croisement de celles-ci avec deux souches haploïdes de la référence CBS 3082 (*MAT*a et *MAT*alpha). Cette dernière étape est cependant désuète pour l'étude à grande échelle de la descendance.

Une méthode est ainsi en cours de développement au sein de l'équipe pour faciliter l'isolement et la sélection des descendants haploïdes dans chaque signe sexuel chez *S. kluyveri*. Les marqueurs fluorescents YFP et mCherry sont placés, dans un même plasmide, sous le contrôle respectivement des promoteurs des gènes *STE2* et *STE3* propres à un signe sexuel chez *S. kluyveri*, à l'aide de la technologie de clonage GATEWAY®. Le gène *STE2* a une expression spécifique aux cellules de signe *MAT*a et le gène *STE3* aux cellules *MAT*alpha. Les cellules haploïdes sont triées par la technique de cytométrie en flux selon leur signe sexuel.

L'approche génétique développée dans ce cadre servira de fondement à des projets à plus grande échelle pour l'étude de la descendance afin de cartographier les bases génétiques de phénotypes en d'autres termes déterminer les origines génétiques (régions, gènes, mutations) impliquées.

Etude de la phosphorylation de l'amphiphysine de levure Rvs167

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Notre laboratoire utilise la **levure *Saccharomyces cerevisiae*** comme modèle d'étude de protéines humaines responsables de **myopathies centronucléaires** (CNM), telles que l'**amphiphysine BIN1**[1]. Son homologue de levure est Rvs167 (Reduced viability upon Starvation167), protéine jouant un rôle dans l'**endocytose** et la **résistance au stress**, via son interaction avec les membranes lipidiques et le cytosquelette d'actine[2,3]. On ne sait pas encore comment la fonction de Rvs167 est régulée. Nous avons identifié un site de phosphorylation en bordure du domaine d'interaction lipidique. Notre objectif est ainsi d'étudier le **rôle de la phosphorylation** de Rvs167 pour sa fonction cellulaire.

Un **mutant de phosphorylation (TA)** et un **mutant phosphomimétique (TD)** ont été obtenus par mutagénèse dirigée sur des plasmides contenant *RVS167-GFP*. Ceux-ci ont ensuite servi à transformer une **souche mutante *rvs167Δ***, qui présente un fort défaut d'endocytose et de résistance au stress salin. Les **phénotypes sauvages sont rétablis** par complémentation avec les protéines Rvs167-GFP et Rvs167-**TA**-GFP, présentes sous forme de **points fluorescents** à la périphérie cellulaire. La protéine mutante Rvs167-**T7A**-GFP se localise davantage sous forme de ces points brillants que la protéine sauvage. Au contraire, la protéine Rvs167-**TD**-GFP ne restaure que partiellement la résistance au stress salin et présente une localisation différente. Ces travaux montrent donc que la phosphorylation de Rvs167 a une influence sur son activité cellulaire.

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New insight in fluorescent *Pseudomonas* iron acquisition in soil: pyoverdine plays a key role in iron oxydes weathering

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Iron is an essential nutrient for most bacteria, required for key biological processes including for example nucleotide synthesis, DNA repair, amino acid synthesis, oxygen transport and respiration. However, iron bioavailability in soil and sediments is low under aerobic conditions because its solubility is controlled by stable hydroxides, oxyhydroxides and oxides (Cornell and Schwertmann, 2003). To overcome the low biodisponibility of iron, a widespread strategy in the microbial communities consists in the production of low-molecular-weight chelating agents, the siderophores. As common inhabitants in soil, fluorescent pseudomonads are characterized by the production of a fluorescent yellow green siderophore, called pyoverdine. This compound, released by bacteria in the environment, has a high affinity for ferric iron (10^{-32} M^{-1}) and therefore could solubilize metal from diverse mineral phases such as clays or iron-(hydr) oxides, abundant in soil. The dissolution of iron-bearing minerals promoted by siderophores has been investigated for some siderophores but has never been focused on pyoverdine. In the present work, we investigated alteration of two iron oxides, a poorly crystallized ferryhydrite and the well crystallized goethite and focused on the role of the siderophore pyoverdine in this process.

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Multiplex titration for eight staphylococcal enterotoxins responsible for food poisoining

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We developed a multiplex assay that allows the simultaneous and specific titration of at least staphylococcal enterotoxins A, B, C, D, E, G, H and I. Any of the cited antigens were expressed in a recombinant form that remains functional and does not harbour significant difference in their antigen epitopes. These antigens are used for immunizations, and serve as controls in the test assays. The titration was based on the Luminex™ technology and uses rabbit polyclonal antibodies that were affinity-purified, and secondarily purified to avoid cross-reactions. This assay reaches mono-specificity with a high sensitivity corresponding to limit of quantification of 50-80pg/g in water, bacterial culture medium or milk. Its process is covered by patent EP-11156131-2.

The multiplex titration finally allows simultaneous specific and quantitative detections with limits lower a bit than the supposed toxic doses and has been evaluated onto contaminated cheeses. Enterotoxins can be detected in a $1/10^6$ molecular ratio with a selectivity range of 96-104% between concentrations from 150 to 10000 pg/mL of dairy matrices. Such a simultaneous titration offers homogeneity and possibilities to detect enterotoxins concentrations that would remain below a secure human toxicity threshold. With the European regulation (CE 1441/2007, December 2007), there is a real need to identify new methods for detection and specific titration of staphylococcal enterotoxins, that overcomes the shortcomings, and some disadvantages of prior art. Thereby, we offer tools improving the management of the eight staphylococcal enterotoxins already associated with food poisoning, while reducing costs and time consumption.

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Staphylococcus aureus Leucotoxins disrupt Ca^{2+} homeostasis in central and sensory neurons through the Store Operated Calcium Entry complex

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Infectious diseases due to *Staphylococcus aureus* include painful symptoms suggesting involvement of the nervous system. In this work we evaluate the potential neurotoxicity of certain virulence factors released by this bacterium. Bi-component leucotoxins trigger the release of Glutamate from primary rat cerebellar granular neurons. Low concentration of leucotoxins causes an increase of free $[Ca^{2+}]_i$, which is responsible of the release of glutamate. Leucotoxins initiated also transient $[Ca^{2+}]_i$ rises in primary sensory neurons of DRG. The $[Ca^{2+}]_i$ imbalance do not depend on the activity of glutamate receptors or voltage operated Ca^{2+} channels but is due to the toxin interaction with the Store Operated Ca^{2+} Entry (SOCE) complex. Drugs targeting the refilling of intracellular Ca^{2+} stores [Sarco-Endoplasmic Reticulum Ca^{2+}-ATPase (SERCA), H^{+}-ATPase] and antagonists of the SOCE complex blunted, or reduced considerably, the leucotoxin induced $[Ca^{2+}]_i$ elevation. Shortly after binding the leucotoxin is quickly internalised, through a Caveolin dependent pathway, and is found in the same endosomal compartment than the Ca^{2+} sensor stromal interacting molecule Stim1. The free internal Ca^{2+} mobilisation is antagonized by specific anti Stim1 antibodies. These data suggest an interaction of leucotoxins with the Ca^{2+} release-activated Ca^{2+} (CRAC) channels and the arachidonic acid-regulated calcium (ARC) channel.

Jover E., Laventie B.J., Tawk M., Poulain B., Prévost G. *Staphylococcus aureus* Leucotoxins Disrupt Ca^{2+} Homeostasis in Central and Sensory Neurons Through the Store Operated Calcium Entry Complex. *Cellular Microbiology*, in press doi: 10.1111/cmi.12069 (2012).

HlgC/HlgB and PVL induce Calcium influx in hPMNs through different pathways

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The gamma-hemolysin HlgC/HlgB and the Panton and Valentine leukocidin are two pore-forming toxins secreted by *Staphylococcus aureus*. These bicomponent leukotoxins bind to the human PMNs cell membrane and form an octameric prepore before the pore formation. The prepores are sufficient to induce a calcium influx regardless to the pore formation. The aim of our study is to identify the calcium entry pathways activated by these leukotoxins by using different inhibitors of calcium channels and immunocytochemistry. The rise of the free intracellular calcium induced by the leukotoxins is investigated by using Fura-2 calcium probe. The structural similarities between these two leukotoxins suggested that their mode of action would be similar (Gauduchon et al, 2001). But surprisingly, the use of the different inhibitors shows that HlgC/HlgB and LPV activate different Ca2+ entry pathways, albeit the ORAI/STIM complex remains involved.

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